510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k042248

B. Purpose for Submission:
New device

C. Measurand:
Acetylcholine receptor autoantibodies

D. Type of Test:
Semi-quantitative, radioimmunoassay (RIA)

E. Applicant:
Kronus Market Development Associates, Inc.

F. Proprietary and Established Names:
Kronus I^{125} Acetylcholine Receptor Antibody Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5660, Multiple Autoantibodies Immunological Test System

2. Classification:
   Class II

3. Product code:
   NST, Autoantibodies, Acetylcholine Receptor, Acetylcholine Blocking and Non-Blocking

4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   The Kronus I^{125} Acetylcholine Receptor Antibody (AChRAb) Assay Kit is for the semi-quantitative determination of acetylcholine receptor antibody in human serum. The AChRAb Assay kit is useful as an aid in the differential diagnosis of Myasthenia Gravis (MG).

2. Indication(s) for use:
   Same as intended use

3. Special conditions for use statement(s):
   The device is for prescription use only.

4. Special instrument requirements:
   Gamma counter for I^{125}

I. Device Description:

The Kronus I^{125} Acetylcholine Receptor Antibody (AChRAb) Assay Kit consists of:

1. Lyophilized I^{125} Acetylcholine Receptor reagent which contains detergent-solubilized fetal and adult acetylcholine receptors extracted from cultures of rhabdomyosarcoma cells and labeled with I^{125} alpha-bungarotoxin.

2. I^{125} AChR diluent – ready-to-use.

3. Negative reference which is normal human serum - ready-to-use.

4. Positive reference which is human serum containing AChR antibody - ready-to-use.

5. Goat anti-human IgG - ready-to-use.

6. Normal human serum for diluting test sera - ready-to-use.

7. Phosphate buffered saline with surfactant - ready-to-use.

J. Substantial Equivalence Information:

The sponsor claimed the new device is substantially equivalent to the Kronus TSH Radioreceptor Antibody (TRAb) Assay Kit (k863006) because both assays use similar methodology. The intended use and indications for use however are different. It was decided that the performance of the new device has to be supported by clinical data.

1. Predicate device name(s):
   Kronus TSH Radioreceptor Antibody (TRAb) Assay Kit

2. Predicate 510(k) number(s):
   K863006

3. Comparison with predicate:

Similarities		
Item	New device	Predicate device
Device Name	Kronus Acetylcholine Receptor Antibody (AChRAb) Assay Kit	Kronus TSH Radioreceptor Antibody (TRAb) Assay Kit
Intended use	Detection of autoantibodies to specific receptor	Same
Test Principle	Radioreceptor immunoassay	Same
Test matrix	Serum	Same
Test Platform	Receptors bind to serum antibodies in a liquid phase (test tube), the antibody-antigen complexes are precipitated and radioactivity measured	Same
Detection instrument	Gamma counter	Same
Differences		
Analyte	Anti-acetylcholine receptor antibodies	Anti-TSH receptor antibodies
Indications for use	Aid in the differential diagnosis of Myasthenia Gravis	Aid in the assessment of patients with Graves' disease
Precipitation reagent	Anti-human IgG	Polyethylene glycol

K. Standard/Guidance Document Referenced (if applicable):

None referenced

L. Test Principle:

Patient specimens and reference reagents are incubated for two hours at room temperature with detergent-solubilized fetal and adult acetylcholine receptors (AChR) labeled with I^{125} –alpha-bungarotoxin. The resulting bound complexes of labeled AChR and autoantibody are then immunoprecipitated with anti-human IgG. After centrifugation, the supernatant is aspirated and the pellet containing labeled AChR/autoantibody-bound complex is counted in a gamma counter. Counts are directly proportional to the amount of autoantibody present and results are expressed as the concentration of alpha-bungarotoxin bound (nmoles/L toxin bound). The assay results are also dependent on the specific activity of the labeled alpha-bungarotoxin, the decay of the labeled toxin-receptor complex in the period between labeling and the day of assay and the gamma counter efficiency.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*

      Intra-assay precision was determined by analyzing 20 replicates of four samples with AChRAb concentrations ranging from 0.97 to 7.24 nmol/L. The following table summarizes the results.

Sample	N	AChRAb		
		Mean concn (nmol/L)	SD (nmol/L)	%CV
1	20	3.3	0.06	1.82
2	20	1.81	0.03	1.66
3	20	0.97	0.03	3.10
4	20	7.24	1.5	2.30

Inter-assay precision was assessed by analyzing a high AChRAb sample (2.2 nmol/L) and a low AChRAb sample (0.50 nmol/L) in 20 runs. Results are shown in table below.

Sample	#Runs	AChRAb		
		Mean concn (nmol/L)	SD (nmol/L)	%CV
1	20	2.2	0.11	5.0
2	20	0.5	0.03	5.9

Additional lot-to-lot and lab-to-lab reproducibility data are provided.
Lot-to-lot precision was determined by analyzing 25 samples with four lots of the Kronus AChRAb kit. The AChRAb concentration ranged from 0 to 18.5 nmol/L. One sample with insufficient volume was only tested on three lots. The %CV ranged from 1.3% to 16.9% with a mean %CV of 6.3%.

Lab-to-lab precision was also determined by analyzing 20 samples in two different laboratories using one lot of Kronus AChRAb kit. The AChRAb concentration ranged from 0.26 to 9.3 nmol/L. The %CV ranged from 0% to 21.8% with a mean %CV of 6.3%.

b. Linearity/assay reportable range:

Linearity – Two-fold dilutions of three low and three high AChRAb samples were tested. The concentrations for the low samples were 1.4, 1.9 and 3.8 nmol/L and 7.6, 14 and 24 nmol/L for the high samples. Results showed the assay is linear within the measuring range.

Recovery study – Eight serum samples with varying acetylcholine receptor levels were spiked with a serum sample containing AChRAb. The percent recoveries ranged from 102% to 118% with a mean recovery of 110% (See results below).

Sample	AChRAb (nmol/L)	Expected AChRab in spiked sample (nmol/L)	Measured AChRAb in spiked sample (nmol/L)	% Recovery
1	4.8	5.44	5.9	108
2	2.5	3.6	3.9	108
3	0.57	2.056	2.4	117
4	6.5	6.8	7.3	107
5	1.4	2.72	3.0	110
6	19.3	17.04	18.4	108
7	8.5	8.4	8.6	102
8	1.4	2.72	3.2	118

The assay measuring range is 0.3 nmol/L to 7.0 nmol/L.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No reference material available.

d. *Detection limit:*
The lower detection limit was determined by sequentially testing the negative control 20 times. The mean and standard deviation (SD) for the cpm were calculated and the mean+2SD and the mean+3SD were converted to nmol/L. The mean+2SD was 0.259 nmol/L and the mean+3SD was 0.269 nmol/L. The lower detection limit was 0.26 nmol/L.

e. *Analytical specificity:*
Interference Study:
Hemoglobin – Ten samples from patients with AChRAb and 5 samples from normal healthy blood donors spiked with hemoglobin at 5 mg/mL were analyzed by the Kronus AChRAb assay. The concentrations of AChRAb in the spiked samples were compared to samples without hemoglobin. Results showed no significant interference at the concentration tested.

Bilirubin – Ten samples from patients with AChRAb and 5 samples from normal healthy blood donors spiked with bilirubin at 20 mg/dL were analyzed by the Kronus AChRAb assay. The concentrations of AChRAb in the spiked samples were compared to samples without bilirubin. Results showed no significant interference at the concentration tested.

Lipids - Ten samples from patients with AChRAb and 5 samples from normal healthy blood donors spiked with Intralipid at 30 mg/dL were analyzed by the Kronus AChRAb assay. The concentrations of AChRAb in the spiked samples were compared to samples without Intralipid. Results showed no significant interference at the concentration tested.

f. *Assay cut-off:*
One hundred and eleven serum samples from human blood donors were

assayed and 96.5% (109) of sera gave results < 0.5 nmol/L. Two samples had AChRAb concentrations of 0.99 nmol/L and 0.5 nmol/L. Based on these results, AChRAb values ≤ 0.2 nmol/L are considered negative, 0.21-0.49 nmol/L are considered equivocal and values ≥ 0.5 nmol/L are considered positive.

2. Comparison studies:
   a. *Method comparison with predicate device:*
   Not applicable since there is no predicate device.

   b. *Matrix comparison:*
   Serum is the only recommended matrix

3. Clinical studies:
   a. *Clinical Sensitivity:*
   Samples from 53 patients with Myasthenia Gravis (MG) with AChRAb concentration ranged from 0.54 to 16.38 nmol/L (mean = 5.44 nmol/L and SD = 4.71 nmol/L) were analyzed using the Kronus AChRAb Kit. A total of 79 samples from other autoimmune diseases which include Graves' Disease (46 samples), Addison's Disease (13 samples), Type I diabetes (10 samples) and Rheumatoid arthritis (10 samples) were also analyzed. All samples were found to be negative except one Graves' Disease sample and one Addison's Disease sample and both samples gave equivocal results (0.28 nmol/L and 0.36 nmol/L respectively). Clinical sensitivity was determined to be 100%.

   b. *Clinical specificity:*
   Based on results on 111 normal healthy donors (as described in Assay Cut-off), the clinical specificity was determined to be 98.2% (109/111).

   c. *Other clinical supportive data (when a. and b. are not applicable):*
   Not applicable.

4. Clinical cut-off:
   See Assay cut-off.

5. Expected values/Reference range:
   The expected value in normal population is less than 0.2 nmol/L.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.